Characterization of Anti-idiotypic Antibodies for High Performance in Bioanalytical Assays

C Löw, R Brundiers, S Harth, M Klee and A Knappik

Bio-Rad Laboratories, Puchheim, Germany

Introduction

Reproducibility and accuracy are essential for successful bioanalytical assay development. The antibodies generated for these assays must be highly specific, of high quality, and supplied to a consistent standard in order to minimize assay variability and ensure confidence in the results. The generation of these critical reagents for ligand binding assays is both cost and labor intensive, so it is important that they are thoroughly characterized and managed to ensure assay integrity.

Here we demonstrate how Bio-Rad applies industry specified standards to characterize recombinant monoclonal anti-idiotypic antibodies that are manufactured and qualified for use in pharmacokinetic (PK) and anti-drug antibody (ADA) assays.

Quality Control

Every new antibody and subsequent batches ('lots') are subject to a strict quality control (QC) procedure. Every new product is first produced in three independent batches. The activity of the batches is compared in a ligand binding assay: ELISA titration for Fab antibodies (Figure 7), ADA bridging ELISA for full immunoglobulin (Ig) antibodies (Figure 1). The batch closest to the average curve is chosen as the future reference batch. Every new batch is compared to the reference batch using the same assay protocol.

Before release, new batches of all antibodies are also routinely tested for specificity, purity (Figures 3 and 4), and Ig antibodies are tested by SEC for monodispersity (Figure 5). In addition, for each new batch production, the antibody gene is re-sequenced to ensure product identity.

For reagents in Ig format, the antibody batches are tested and compared using an ADA bridging ELISA set-up. Figure 1 shows batch consistency with an anti-palivizumab antibody as an example.

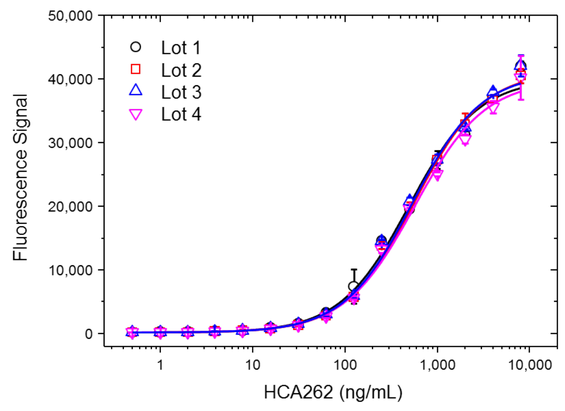
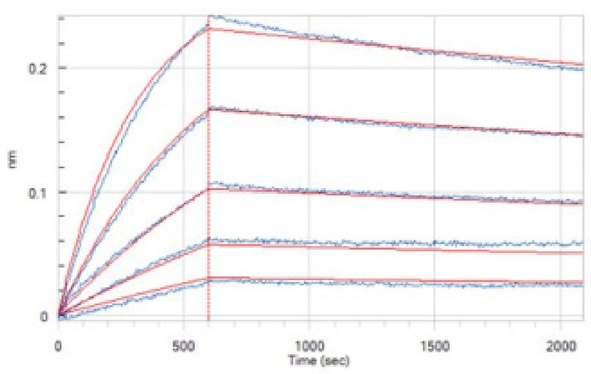


Fig. 1. Batch to batch consistency. A microtiter plate was coated over night with palivizumab (1 µg/ml). After washing and blocking with PBST+5% BSA, PBST with 10% human serum, spiked with increasing concentrations of human anti-palivizumab antibody (HCA262, Lot 1-4) was added. Detection was performed using HRP conjugated palivizumab (2 µg/ml) in HISPEC assay diluent (BUF049A) and QuantaBlu™ fluorogenic peroxidase substrate. Data are shown as the mean of three measurements.

Affinity Measurement

Affinity is the strength of binding between an antigen and an antibody binding site. The equilibrium dissociation constant between the antibody and its antigen is designated K_D and is the ratio of the experimentally measured off- and on- rates, k_d and k_a, respectively. The lower the K_D value, the higher the affinity of the antibody. The affinity of all anti-biotherapeutic antibodies in their monovalent format is measured using biolayer interferometry. An example is shown below for an inhibitory anti-idiotypic antibody (Figure 2). The monovalent Fab format for affinity determination is used, as the measured affinity truly reflects the intrinsic antigen-binding affinity due to the monovalent interaction with the antigen.



Antibody ID	k_a	k_d	K_D
	[1/Ms]	[1/s]	[nM]
HCA265	4.44E+05	1.13E-04	0.3

Fig. 2. Affinity determination for an inhibitory (Type 1) anti-idiotypic antibody. 10 µg/ml panitumumab in 10 mM sodium acetate pH 4 was immobilized on Octet® RED384 (Pall FortéBio) AR2G sensors with a coating density of 1.4 +/- 0.1 nm. The human anti-panitumumab antibody (HCA265), in a monovalent Fab format was then perfused over the sensor surface using a 1:2 dilution series with a starting concentration of 6.25 nM. An additional sensor perfused with experimental buffer only was used as reference for compensating any baseline drifts. Sensors were regenerated after each cycle with 10 mM glycine pH 2.0. Experimental buffer was PBS pH 7.4, 0.1% (w/v) BSA and 0.02% (v/v) Tween®-20. The experiment was performed at 30˚C with a shake speed of 1000 rpm. A 1:1 interaction model was used to fit the data using the FortéBio Data Analysis software 8.2.0.7. Measured data are shown in blue; interaction fit is shown in red. Calculated association and dissociation rates and the K_D value are given in the table.

Purity Assessment - Fab Antibodies

Purity of every new batch of Fab antibodies is assessed using SDS PAGE with subsequent Coomassie staining (Figure 3).

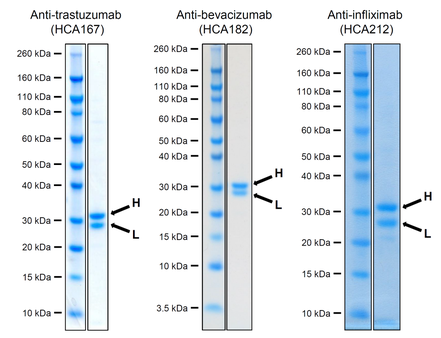


Fig. 3. Purity assessment of Fab format antibodies. Human anti-trastuzumab (HCA167), anti-bevacizumab (HCA182) and anti-infliximab (HCA212) Fab antibodies were analyzed by SDS-PAGE (2 µg per lane). Heavy chains (H) and light chains (L) are visible at ~32 kDa and ~28 kDa, respectively.

Purity Assessment - Full Length Immunoglobulins

Purity of Ig antibodies is assessed using capillary electrophoresis (CE) (Figure 4). Size exclusion chromatography (SEC) is used to look for the presence or absence of aggregates (Figure 5).

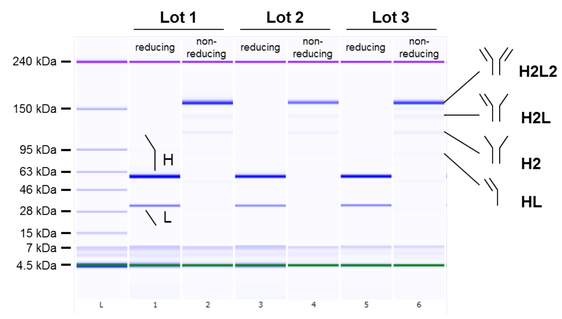
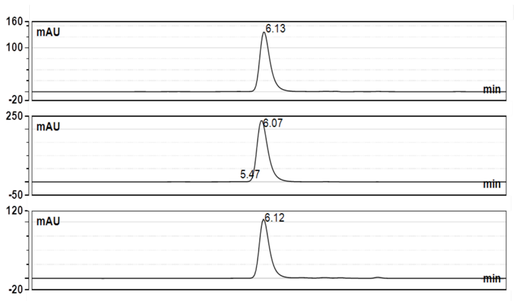


Fig. 4. Purity assessment of Ig format antibodies. Three different batches of human anti-palivizumab IgG (HCA262, Lot 1-3) were analyzed by CE. Samples were denatured and run under reducing and non-reducing conditions. Heavy chains (H) and light chains (L) under reducing denaturing conditions are visible at ~50 kDa and ~30 kDa, respectively. Intact IgG molecules (H2L2) are visible at ~150 kDa under non-reducing denaturing conditions.



	#	Peak Name	Retention Time (min)	Rel. Area [%]
Lot 1	-	Aggregates	-	-
	1	hIgG	6.132	100.00
Lot 2	#	Peak Name	Retention Time (min)	Rel. Area [%]
	1	Aggregates	5.465	0.59
	2	hIgG	6.065	99.41
Lot 3	#	Peak Name	Retention Time (min)	Rel. Area [%]
	-	Aggregates	-	-
	1	hIgG	6.115	100.00

Fig. 5. SEC analysis. Three different batches of human anti-palivizumab IgG (HCA262, Lot 1-3) were analyzed by SEC.

Stability

Antibody stability can be tested by using an accelerated stability testing protocol or by monitoring activity after several freeze-thaw cycles. In the example below, the anti-bevacizumab antibody HCA185 (hIgG1) was tested by measuring activity after up to 14 days incubation at 37˚C.

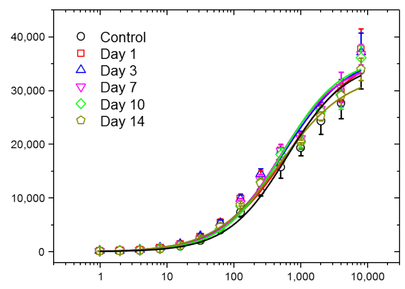


Fig. 6. Accelerated stability test. Freshly thawed human anti-bevacizumab IgG (HCA185) was diluted to 1 mg/ml, aliquoted and placed at 37˚C. Aliquots were removed from 37˚C at every time point and placed at 4˚C along with the control. A microtiter plate was coated over night with bevacizumab (1 µg/ml). After washing and blocking with PBST+5% BSA, PBST with 10% human serum, spiked with increasing concentrations of anti-bevacizumab (HCA185) was added. Detection was performed using HRP conjugated bevacizumab (2 µg/ml) in HISPEC assay diluent (BUF049A) and QuantaBlu fluorogenic peroxidase substrate. Data are shown as the mean of three measurements.

Two independent batches of the anti-adalimumab antibody HCA202 (Fab format) were tested for activity after several freeze-thaw cycles using indirect ELISA, Figure 7.

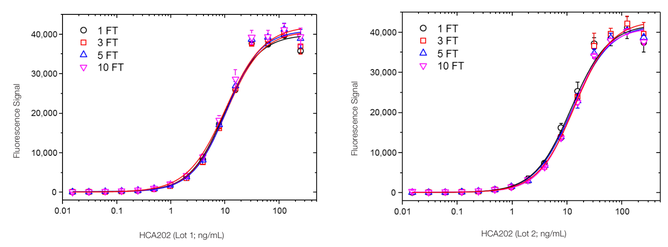


Fig. 7. Freeze-thaw test for Fab format. Two lots of human anti-adalimumab Fab-FH antibody (HCA202) were subjected to the indicated number of freeze-thaw cycles (FT). A microtiter plate was coated over night with adalimumab (5 µg/ml). After washing and blocking with PBST+5% BSA, PBST with 10% human serum, spiked with increasing concentrations of human anti-adalimumab Fab-FH antibody was added. Detection was performed using HRP conjugated anti-His-tag antibody (MCA5995P) at a dilution of 1:2000 in HISPEC assay diluent (BUF049A) and QuantaBlu fluorogenic peroxidase substrate. Data are shown as the mean of three measurements.

bio-rad-antibodies.com/biotherapeutics

UK & Worldwide
Tel: +44 1865 852 700
antibody_sales_uk@bio-rad.com

Europe
Tel: +49 89 80 90 95 21
antibody_sales_de@bio-rad.com

Americas
Toll Free: +1 800 265 7376 (USA & Canada)
Tel: +1 919 876 7978
antibody_sales_us@bio-rad.com